Spatial and Diel Variability in Photosynthetic and Photoprotective Pigments in Shallow Benthic Communities

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Award #N000149910130

LONG-TERM GOALS

Our overall goal is to understand how photosynthetic and photoprotective pigments in benthic plants (primarily benthic microalgae) affect the optical properties (primarily spectral reflectance and fluorescence) of shallow benthic environments. The information gained will be used for the development and testing of rapid scanning optical techniques for detecting and assessing changes and specific disturbances in benthic communities.

OBJECTIVES

Our main objective is to determine the spatial and temporal (particularly diel) variation in a variety of photosynthetic and photoprotective pigments and examine how these pigments affect the spectral reflectance and fluorescence at the sediment surface. An understanding of these relationships is needed in order to refine algorithms used for processing data collected with various multispectral and hyperspectral imaging instruments used for identification and characterization of both living and man-made objects in shallow benthic environments.

APPROACH

Sediment cores with overlying water were collected at various sites around Lee Stocking Island, Bahamas and transported back to the field lab within hours. Spectral fluorescence and reflectance was measured back in the field lab at the surface of the cores while inside a light-tight container. Spectral fluorescence was measured with a SPEX Fluorolog-3 spectrofluorometer with a fiber optic probe held 10 mm from the sediment surface with a X-Y-Z positioner, and with an Ocean Optics S2000-FL fluorometer with a fiber optic probe held 5 mm from the sediment surface. Spectral reflectance was measured with an Ocean Optics S2000 UV-VISspectrometer with a fiber optic probe held perpendicular and 25 mm from the sediment surface. The surfaces of sediments cores were also

Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
30 SEP 2001		**00-00-2001 to 00-00-2001**

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
Spatial and Diel Variability in Photosynthetic and Photoprotective Pigments in Shallow Benthic Communities	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **Rosenstiel School of Marine and Atmospheric Science,,University of Miami,4600 Rickenbacker Cswy,,Miami,,FL, 33149**	8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
Our overall goal is to understand how photosynthetic and photoprotective pigments in benthic plants (primarily benthic microalgae) affect the optical properties (primarily spectral reflectance and fluorescence) of shallow benthic environments. The information gained will be used for the development and testing of rapid scanning optical techniques for detecting and assessing changes and specific disturbances in benthic communities.

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a REPORT	b ABSTRACT	c THIS PAGE			
unclassified	**unclassified**	**unclassified**	**Same as Report (SAR)**	**6**	

examined and photographed with an Olympus SZX-12 stereo microscope to document grain characteristics, microalgal communities, and extracellular polymeric substances at the sediment surface.

Radiance reflectance (R) was calculated relative to a calibrated WS-1 Spectralon (LabSphere) diffuse reflectance standard (99% reflective). Raw radiance reflectance data were smoothed using a cubic spline, pre-averaged with 60 nodes and a number of points equal to the original data set (2048 points). Subsequently, 1^{st} and 2^{nd} derivatives of reflectance spectra were calculated using 7 nm intervals. Second derivative spectra were used, along with published in-vivo absorption peaks for individual pigments, to identify wavelengths in the reflectance spectra that were most affected by specific pigments. The 1^{st} derivative (slope) for a 7 nm waveband which corresponded to absorption by a specific pigment was divided by radiance reflectance at the wavelength most affected by that pigment (as identified by 2^{nd} derivative analysis). This normalization of 1^{st} derivatives to reflectance was necessary to correct for decreases in reflectance across the spectrum caused by varying amounts of extracellular polysaccharides (EPS) produced by the microalgae at different sampling sites.

For pigment analyses, the top 5 mm of the cores were removed and frozen in liquid nitrogen. For chlorophyll analysis, the sediments were extracted repeatedly with 90% acetone until little pigment was observed in the last extract. Fluorescence of the samples was measured before and after acidification with a Turner 10-000R fluorometer. For phycoerythrin and phycocyanin analysis, the sediments were extracted repeatedly with a phosphate buffer. An ultrasonicator was used to extract pigments. Concentrations were determined with a SPEX Fluorolog-3 spectrofluorometer calibrated with pure pigments obtained from Sigma Chemical Co. Chlorophylls a, b, and c were also determined with the SPEX spectrofluorometer. For chlorophyll and carotenoid analysis, the cores were extracted in 100% acetone and analyzed using a Hewlett Packard High Performance Liquid Chromatograph attached to a diode array detector. Photosynthetic and photoprotective pigments were identified and quantified by comparing with standards purchased from VKI.

For examination of diel variation, cores were incubated in outdoor water tables with flowing seawater. At various times of the day and night, these cores were placed in a light tight box and their spectral fluorescence and spectral reflectance measured using a fiber optic probe as above. For in situ fluorescence measurements, a WET Labs ECO-DFLB underwater fluorometer was placed in light tight cradles implanted in the sediments at various times of the day. The cradles were designed so that the sediment surface received natural sunlight except for the 2 minutes when the fluorometer was placed in the cradle. In addition to fluorescence of algae at the sediment surface, in vivo fluorescence was also measured in the water column of large concrete 1 meter deep aquaculture tanks on a diel basis for comparison.

WORK COMPLETED

We have completed our field sampling and all of our sample processing for years 1-4. Analysis of samples collected in April, 2001 is continuing. Analysis and comparison of all the data is ongoing.

RESULTS

Considerable spatial variation exists at various locations. At Lee Stocking Island in the Bahamas, ooids have chlorophyll concentrations around 10-15 mg m^{-2}. Freshly excavated shrimp mounds also have low chlorophyll concentrations in this range. Offshore, calcareous sands in North Perry reef

ranged from 11 to 25 mg m^{-2} but offshore muds in the area had chlorophyll concentrations around 33-41 mg m^{-2}. A variety of inshore sediments around Lee Stocking Island had chlorophyll concentrations ranging from 14 to 68 mg m^{-2} The areas around Lee Stocking Island with the largest benthic microalgal biomass were an area with grapestone development, with chlorophyll concentrations of 73-104 mg m^{-2}, and Norman's Pond with concentrations of 57-177 mg m^{-2}. Phycoerythrin:chlorophyll and phycocyanin:chlorophyll ratios exhibit a wide range, indicating the proportion of prokaryotic cyanobacteria to eukaryotic algae varies considerably in the sediment surface of these habitats.

Among 66 core samples taken in April, 2001, there was approximately a 10-fold range in concentrations of chlorophyll a among the different habitats, as has been observed in previous years. Chlorophyll c, on the other hand, exhibited a 300-fold range, indicating considerable variation in species composition. While phycoerythrin concentrations varied around 20-fold, phycocyanin varied approximately 70-fold. The highest levels of chlorophylls a and c, and phycocyanin were found in grapestone sediments and the lowest levels of chlorophylls a and c were found in ooid sands. Considerable variation in species composition is reflected in the wide range of pigment ratios: 46 for chl c/chl a; 94 for phycoerythrin/chl a; and 27 for phycocyanin/chl a.

First derivatives of spectral reflectance and pigments concentrations of 152 samples collected in 18 environments around LSI were used to estimate the contributions of specific pigments to decreases in reflectance at specific (7 nm) wavebands. Linear regression of chlorophyll a plus chlorophyllide a (normalized to chlorophyll a based on their extinctions coefficients) concentrations versus the ratio of the 1st derivative (662 nm):reflectance (676 nm) indicates that approximately 70% of the decrease in reflectance around 676 nm can be explained by these pigments (Fig. 1). It should be noted that if chlorophyllide a is not normalized to chlorophyll a, the estimate decreases to 67 %. Likewise, combined concentrations of fucoxanthin and peridinin account for approximately 70% of the decrease in reflectance at 538 nm (Fig. 2).

As observed and reported before, we observed in ten incubated cores in April, 2001 a large variation in chlorophyll fluorescence over the diel cycle (Fig. 3). No significant diel variation is observed in spectral reflectance (measured in different ways in Figs. 4 and 5). This suggests that chlorophyll was constant over the diel cycle and all the fluorescence variation is due to photoadaptation. Specifically, nonphotochemical quenching can probably explain the large drop in chlorophyll fluorescence during the day with no significant change in chlorophyll concentration.

IMPACT/APPLICATIONS

These data indicate that it should be possible to characterize benthic microalgal communities using remote sensing. Fluorescence techniques need to take into account the strong diel cycle in fluorescence. Our data in the Bahamas show no strong diel variation in the chlorophyll absorption.

TRANSITIONS

The information gained from these studies is being used by other CoBOP investigators to create spectral libraries of different habitat types and for comparison with PHILLS images and reflectance measurements made with tethered spectral reflectance buoys and other moored instruments.

RELATED PROJECTS

In a project funded by NOAA, we are examining the spatial and temporal distribution of benthic microalgae in Florida Bay and their relationship to nutrient and phytoplankton distributions.

PUBLICATIONS

Brand, L.E. 2000. Spatial and temporal variation in the optical properties of benthic microalgae. Ocean Optics XV (abstract).

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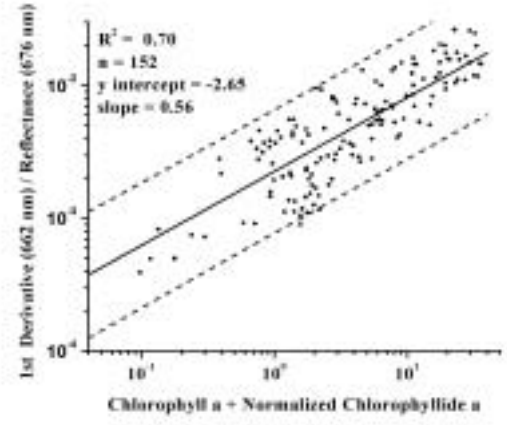

Fig. 1. Relationship between reflectance and chlorophyll a + chlorophyllide a in sediments. Dashed lines, 95% prediction interval.

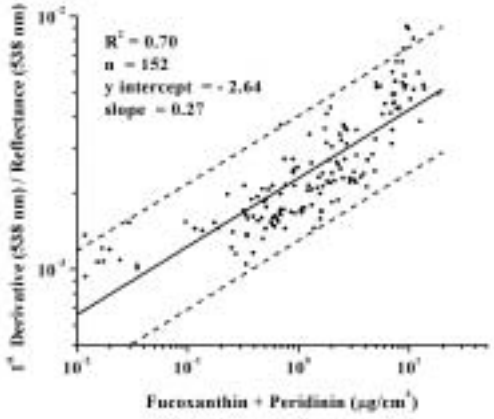

Fig. 2. Relationship between reflectance and fucoxanthin + peridinin in sediments. Dashed lines, 95% prediction interval.

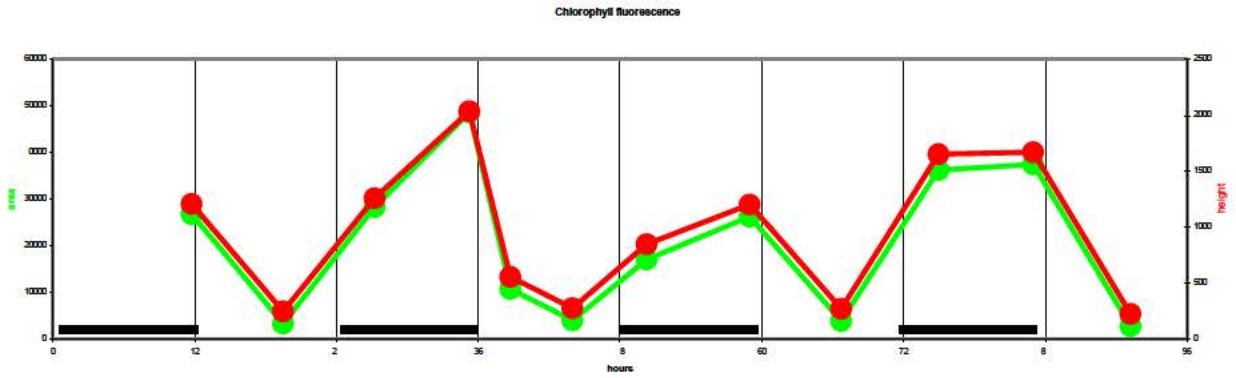

Figure 3. Area and height of chlorophyll fluorescence peak measured with an Ocean Optics S2000-FL fluorometer.

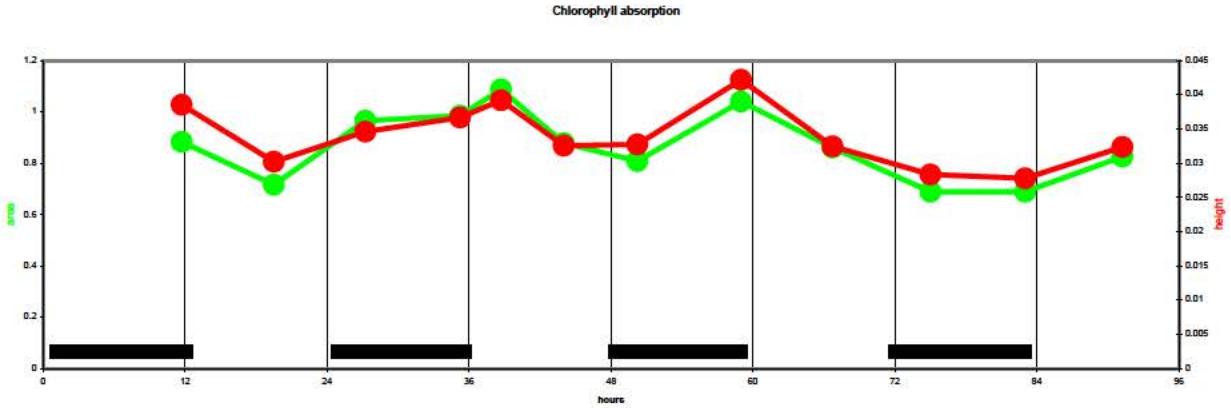

Figure 4. Area and height of chlorophyll absorption dip in reflection spectra measured with an Ocean Optics S2000 UV-Vis spectrometer.

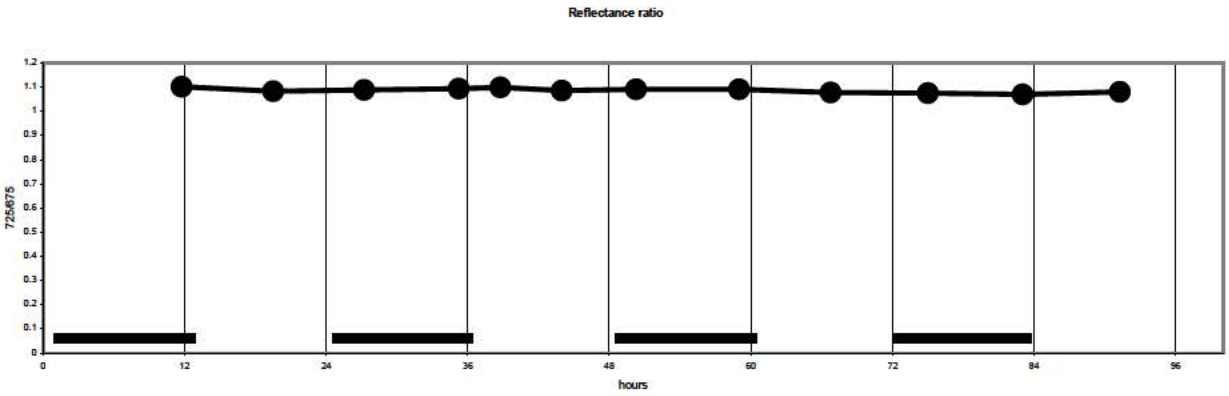

Figure 5. Ratio of reflectance at 725 nm and 675 nm measured with an Ocean Optics S2000 UV-Vis spectrometer.